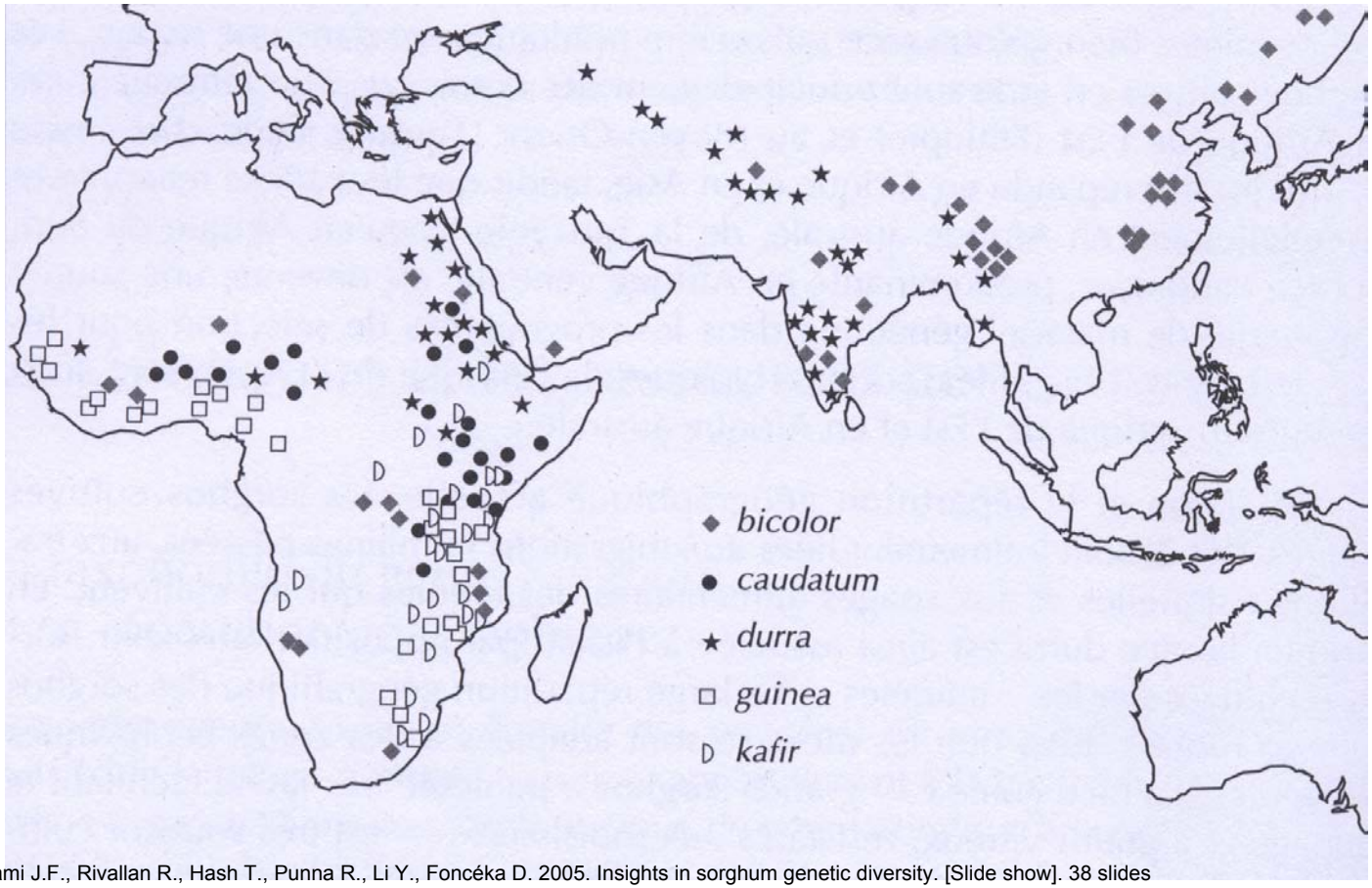
Presentation of Sorghum

- 5th cereal in the world (43 M ha, 62 M T)
- Mainly in Africa and Asia



Billot C., Deu M., Rami J.F., Rivallan R., Hash T., Punna R., Li Y., Foncéka D. 2005. Insights in sorghum genetic diversity. [Slide show]. 38 slides Annual Research Meeting Generation Challenge Program, 2005/09/29-2005/10/01, Rome, Italie.

Sorghum

1st year PI: C. Billot (CIRAD)

2nd year PI: T; Hash (ICRISAT)

Collaborators: CAAS (Yu LI)

Billot C., Deu M., Rami J.F., Rivallan R., Hash T., Punna R., Li Y., Foncéka D. 2005. Insights in sorghum genetic diversity. [Slide show]. 38 slides Annual Research Meeting Generation Challenge Program, 2005/09/29-2005/10/01, Rome, Italie.

Classification

Family : Poaceae
Subfamily: panicoïdeae
Tribe: andropogoneae (like sugarcane, maize….)
Genus: *Sorghum*

5 sections including *Sorghum*

3 crossing species

- *S. halepense* (tetraploide)
- *S. propinquum* (diploïd)
- *S. bicolor* (diploïd)
  - ssp. *drumondii*
  - ssp. *arundinaceum*
  - ssp. *bicolor*

] Wild, perennial

Cultivated, annua

5 races : bicolor
caudatum
durra
guinea
kaffir

10 intermediates (ex: durra-caudatum)

2n=2x=20
750 Mb
Highly self pollinated

Billot C., Deu M., Rami J.F., Rivallan R., Hash T., Punna R., Li Y., Foncéka D. 2005. Insights in sorghum genetic diversity. [Slide show]. 38 slides Annual Research Meeting Generation Challenge Program, 2005/09/29-2005/10/01, Rome, Italie.

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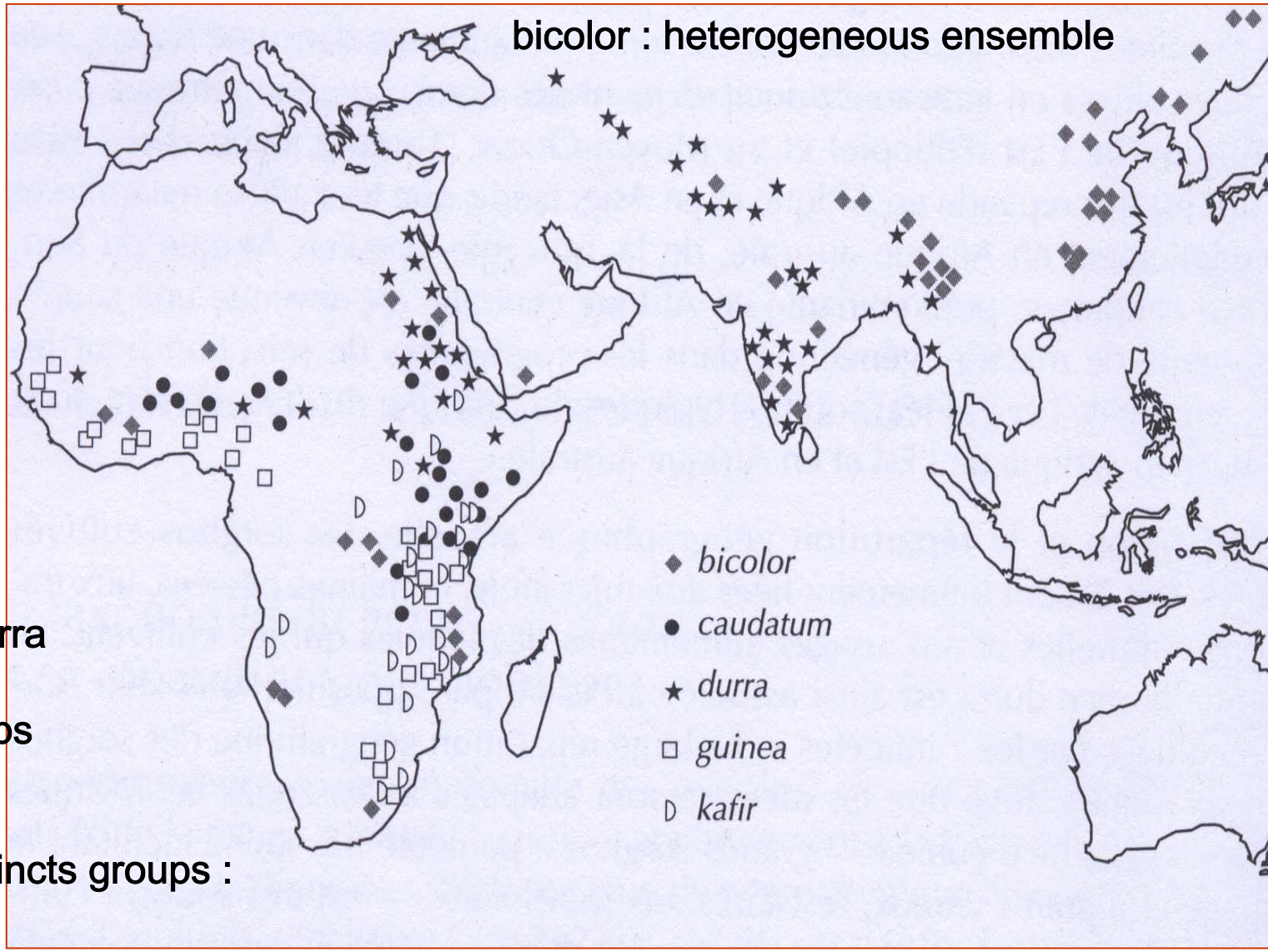
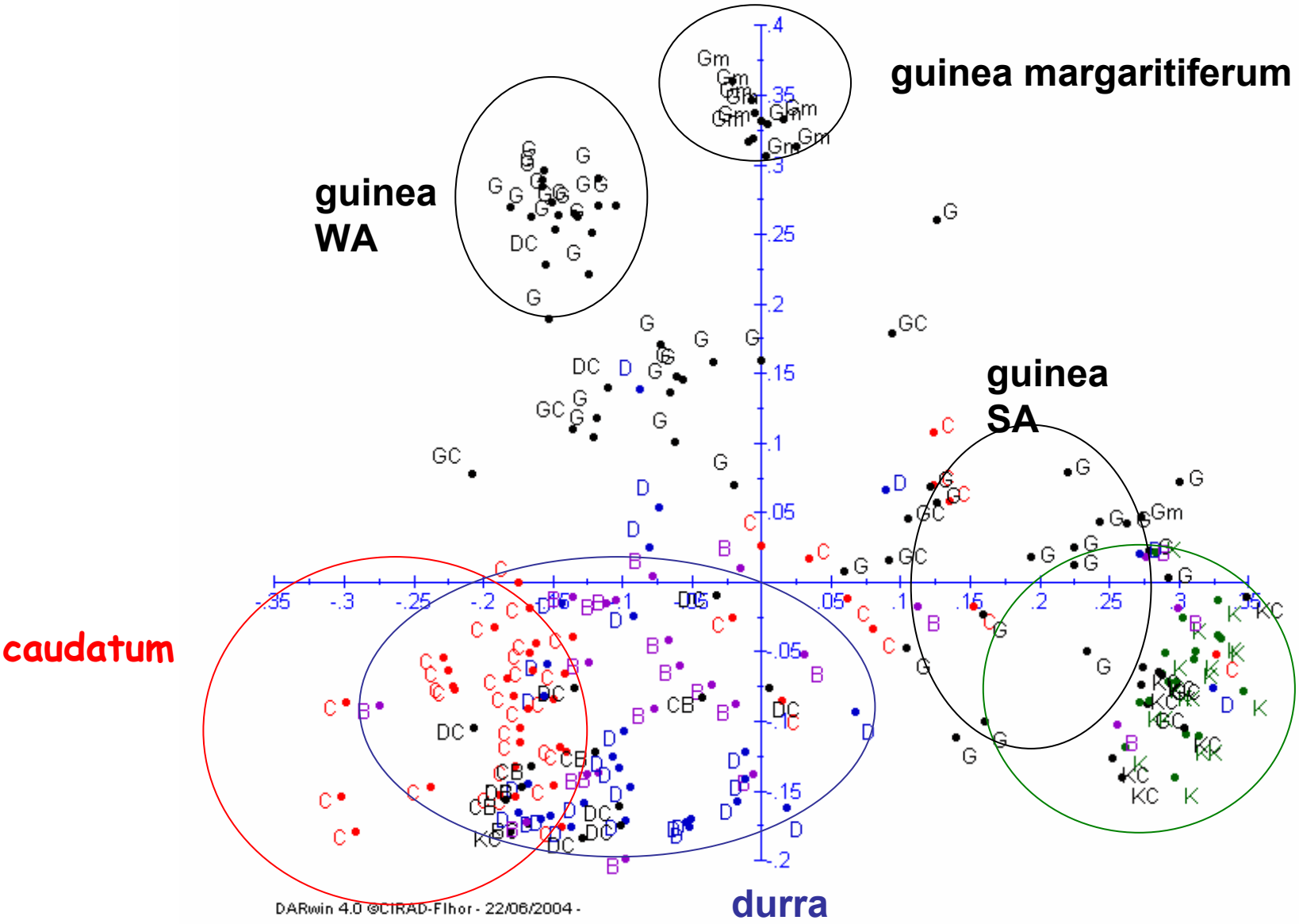


caudatum, durra and kafir: distincts groups

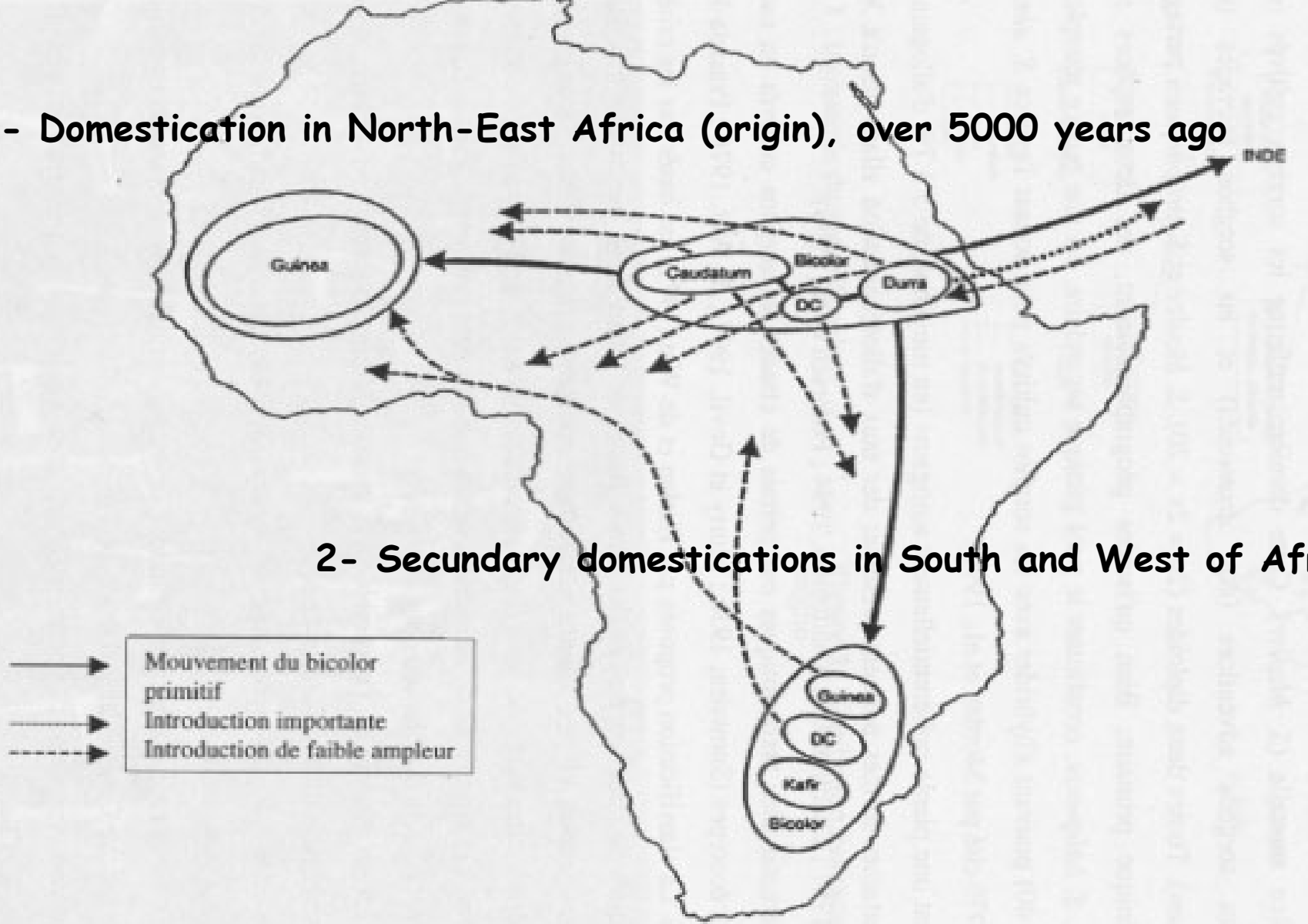
guinea : 3 distincts groups :
- South Africa
- West Africa
- guinea margaritiferum

Billot C., Deu M., Rami J.F., Hamon P., Mash T., Punna R., Li Y., Foncéka D. 2005. Insights in sorghum genetic diversity. [Slide show]. 38 slides Annual Research Meeting Generation Challenge Program, 2005/09/29-2005/10/01, Rome, Italie.



Billot C., Deu M., Rami J.F., Rivallan R., Hash T., Punna R., Li Y., Foncéka D. 2005. Insights in sorghum genetic diversity. [Slide show]. 38 slides Annual Research Meeting Generation Challenge Program, 2005/09/29-2005/10/01, Rome, Italie.

1- Domestication in North-East Africa (origin), over 5000 years ago



2- Secundary domestications in South and West of Afri

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Sorghum collections

- ICRISAT: 36,774 accessions from 91 countries, consisting of landraces, improved breeding lines, cultivars, genetic stock, and the wild relatives.
- CAAS: 16,874 accessions from 34 countries, 90% being landraces
- Agropolis collections are composed of :
  - 2200 accessions of CIRAD collection
  - 3600 accessions of IRD collection coming from west african propections
- Western Africa NARS:
  - Mali: 1300 local ecotypes already evaluated for agro-morphological traits in US and Mali
  - Niger: 540 local ecotypes
  - Senegal: about 200 local ecotypes
  - Burkina Faso: 600 local ecotypes with ongoing characterization
- East Africa
  - Erythrea : 600 accessions not included in ICRISAT collection
  - Sudan: 2300 accessions
- Brasil Collection (Embrapa)

Billot C., Deu M., Rami J.F., Rivallan R., Hash T., Portères R., Li Y., Foncéka D. 2005. Insights in sorghum genetic diversity. [Slide show]. 38 slides Annual Research Meeting Generation Challenge Program, 2005/09/29-2005/10/01, Rome, Italie.

SP1C1 - Selection of accessions

- Selection of 48 accessions
  Largest diversity to test for markers
  Agropolis-CIRAD

- Selection of 700 accessions
  To be genotyped during 1st year
  – CAAS : 250 (<China)
  – Agropolis-Cirad : 250 (previous core-collection + completion)
  – ICRISAT : 200 (completion from international collection)
  – Criteria
    - Collections already studied
    - Diversity
    - References

  46 % from Asia (from which 86% are from China)
  10 % from East of Africa
  13 % from West of Africa
  12 % from South of Africa
  11 % are breeding lines

- Selection of 2300 accessions
  Same criteria, previous study + completion of previous 700 accessions
  To be genotyped during 2nd year

Billot C., Deu M., Rami J.F., Ruivenkan P.V., Hash T., Punna R., Ji Y., Rhoncéka D. 2005. Insights in sorghum genetic diversity. [Slide show]. 38 slides Annual Research Meeting Generation Challenge Program, 2005/09/29-2005/10/01, Rome, Italie.

SP1C2 - Genetic Diversity

- Task 1- Genotyping of 700 accessions
  - Selection of 100 SSR markers
  - Genotyping of 48 accessions in each lab
  - Comparing the results and choosing the set of 50 markers
  - Genotyping of 700 accessions with 30 markers

  CAAS, ICRISAT, Agropolis-CIRAD

- Task 2- Selection of EST-derived SSR

  ICRISAT

- Task 3- Identification of new genomic markers

  CAAS, Agropolis CIRAD

Billot C., Deu M., Rami J.F., Rivallan R., Hash T., Punna R., Li Y., Foncéka D. 2005. Insights in sorghum genetic diversity. [Slide show]. 38 slides Annual Research Meeting Generation Challenge Program, 2005/09/29-2005/10/01, Rome, Italie.

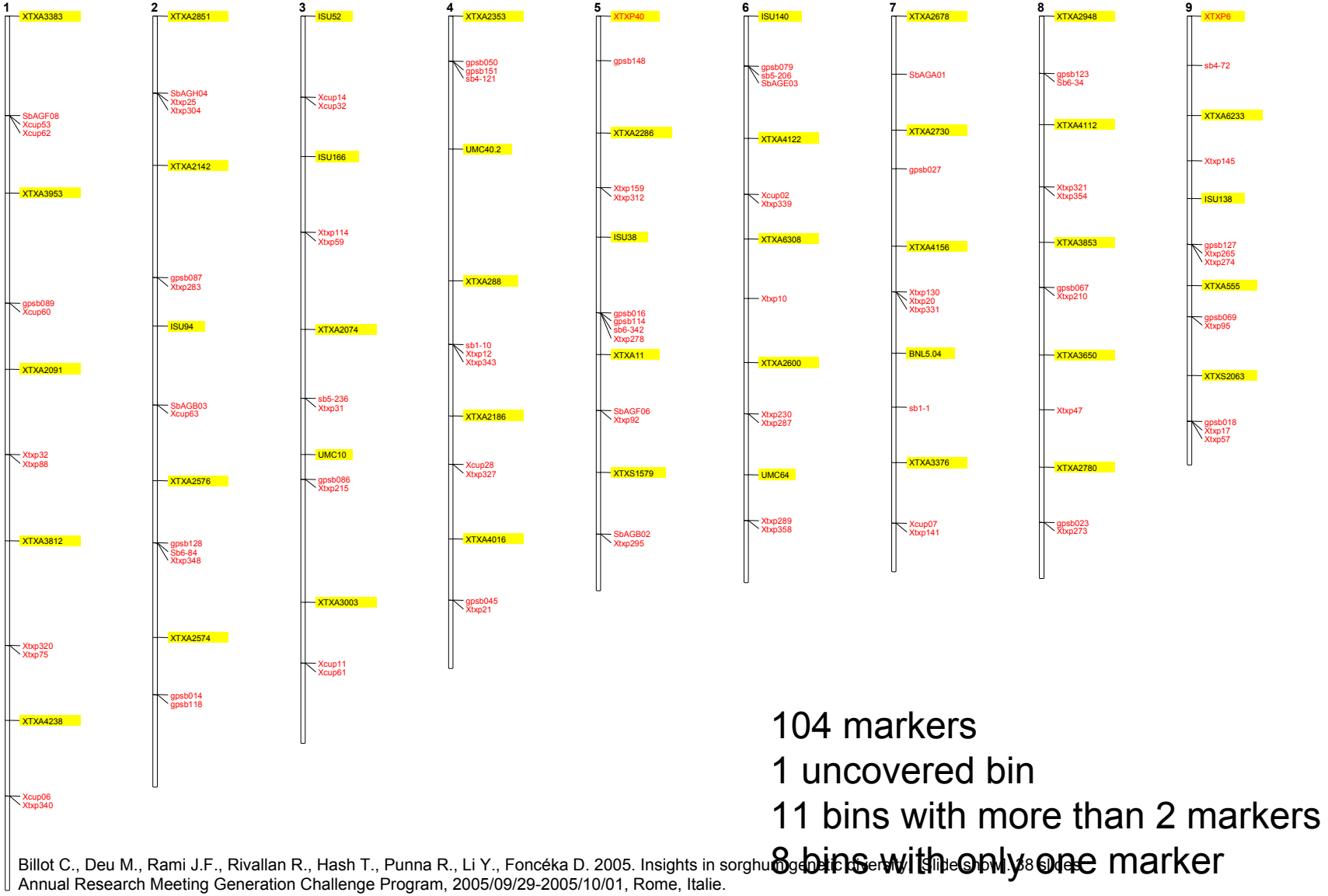
Choice of 100 markers

- 5 bins per chromosome
- 2 SSR/bin
- choice criteria
  - availability of primers in at least one lab
  - tri-nucleotide vs di-nucleotide SSR pattern
  - quality of amplification when known
  - when no marker was available for a bin, one additional marker was chosen in adjacent bins.
  - 26 markers from ICRISAT mandatory

- Results: 104 SSR markers
  - 66 dinucleotides
  - 31 trinucleotides
  - 5 tetranucleotides
  - 1 pentanucleotide
  - 1 hexanucleotide

Billot C., Deu M., Rami J.F., Rivallan R., Hash T., Punna R., Li Y., Foncéka D. 2005. Insights in sorghum genetic diversity. [Slide show]. 38 slides Annual Research Meeting Generation Challenge Program, 2005/09/29-2005/10/01, Rome, Italie.

Choice of 100 markers



104 markers
1 uncovered bin
11 bins with more than 2 markers
8 bins with only one marker

Billot C., Deu M., Rami J.F., Rivallan R., Hash T., Punna R., Li Y., Foncéka D. 2005. Insights in sorghum genetic diversity. Slideshow. 38 Slides. Annual Research Meeting Generation Challenge Program, 2005/09/29-2005/10/01, Rome, Italie.

Choice of 30 markers

- Genotyping of 48 accessions in each lab
  - CAAS :
    - ABI, capilary system
    - M13 tail
    - Genescan and Genotyper softwares
  - ICRISAT
    - ABI, capilary system
    - Labeled primers
    - Genescan and Genotyper softwares
  - Agropolis-CIRAD
    - LICOR, gel system
    - M13 tail
    - SAGA GT software

- Performed in june 2004

Billot C., Deu M., Rami J.F., Rivallan R., Hash T., Punna R., Li Y., Foncéka D. 2005. Insights in sorghum genetic diversity. [Slide show]. 38 slides Annual Research Meeting Generation Challenge Program, 2005/09/29-2005/10/01, Rome, Italie.

Choice of 30 markers

- Criteria for choice amond the 3 datasets
  - hererozygous correctly scored
    - number of differences
  - stable difference in size (taking into account the M13 tail)
    - correlation coefficient
    - SD
- Results
  - ***Parameters:***
    - Hetero Threshold 2
    - Corr Threshold 0.9
    - SD Threshold 1
  - ***Results:***
    - Number of markers OK 27
    - Number of markers OK for 3 comparisons 1
    - Number of markers OK for 2 comparisons 3
    - Number of markers OK for 1 comparisons 23

Billot C., Deu M., Rami J.F., Rivallan R., Hash T., Punna R., Li Y., Foncéka D. 2005. Insights in sorghum genetic diversity. [Slide show]. 38 slides Annual Research Meeting Generation Challenge Program, 2005/09/29-2005/10/01, Rome, Italie.

Not satisfactory

Criteria ?

- hererozygous correctly scored
- stable difference in size (taking into account the M13 tail)
- « subjective » appreciation of the marker quality

12 markers chosen by Agropolis-Cirad
20 markers chosen by ICRISAT

Parameters:

Hetero Threshold:	2
Corr Threshold:	0.9
SD Threshold:	1

Results:

Number of markers OK	27
Number of markers OK for 3 comparisons	1
Number of markers OK for 2 comparisons	3
Number of markers OK for 1 comparisons	23
Total number of markers (including those already proposed by Icrisat or Cirad)	48
Number of uncovered bins (on a total of 49 bins)	12
Number of bins with more than 1 marker	11
Max number of marker in a bin	2

Billot C., Deu M., Rami J.F., Rivallan R., Hash T., Punna R., Li Y., Foncéka D. 2005. Insights in sorghum genetic diversity. [Slide show]. 38 slides Annual Research Meeting Generation Challenge Program, 2005/09/29-2005/10/01, Rome, Italie.

Criteria ?

- hererozygous correctly scored
- stable difference in size (taking into account the M13 tail)
- « subjective » appreciation of the marker quality

12 markers chosen by Agropolis-Cirad -1
20 markers chosen by ICRISAT

1- Verification one by one of each of the selected markers

Criteria of validation : verification of the gels
good thresholds (heterozygous, Corr, SD)

Cirad markers : 7 out of 12
ICRISAT markers : 11 out of 20 ⟶ 17 different loci

2- Verification of automatic selection

H threshold: =<2
Corr threshold: >0.9
SD threshold: =<1

12 new markers, verified

Billot C., Deu M., Rami J.F., Rivallan R., Hash T., Punna R., Li Y., Foncéka D. 2005. Insights in sorghum genetic diversity. [Slide show]. 38 slides
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⟶ **34 markers**

Optimisation of experiment

- Size ladder composed of alleles
  - Selection of 10 DNA (<48) representing high level of variability
  - Used as 3 control: pooled DNA used for each PCR and run
- Necessity of using controls
- Multiplexing (size + dye)

Billot C., Deu M., Rami J.F., Rivallan R., Hash T., Punna R., Li Y., Foncéka D. 2005. Insights in sorghum genetic diversity. [Slide show]. 38 slides Annual Research Meeting Generation Challenge Program, 2005/09/29-2005/10/01, Rome, Italie.

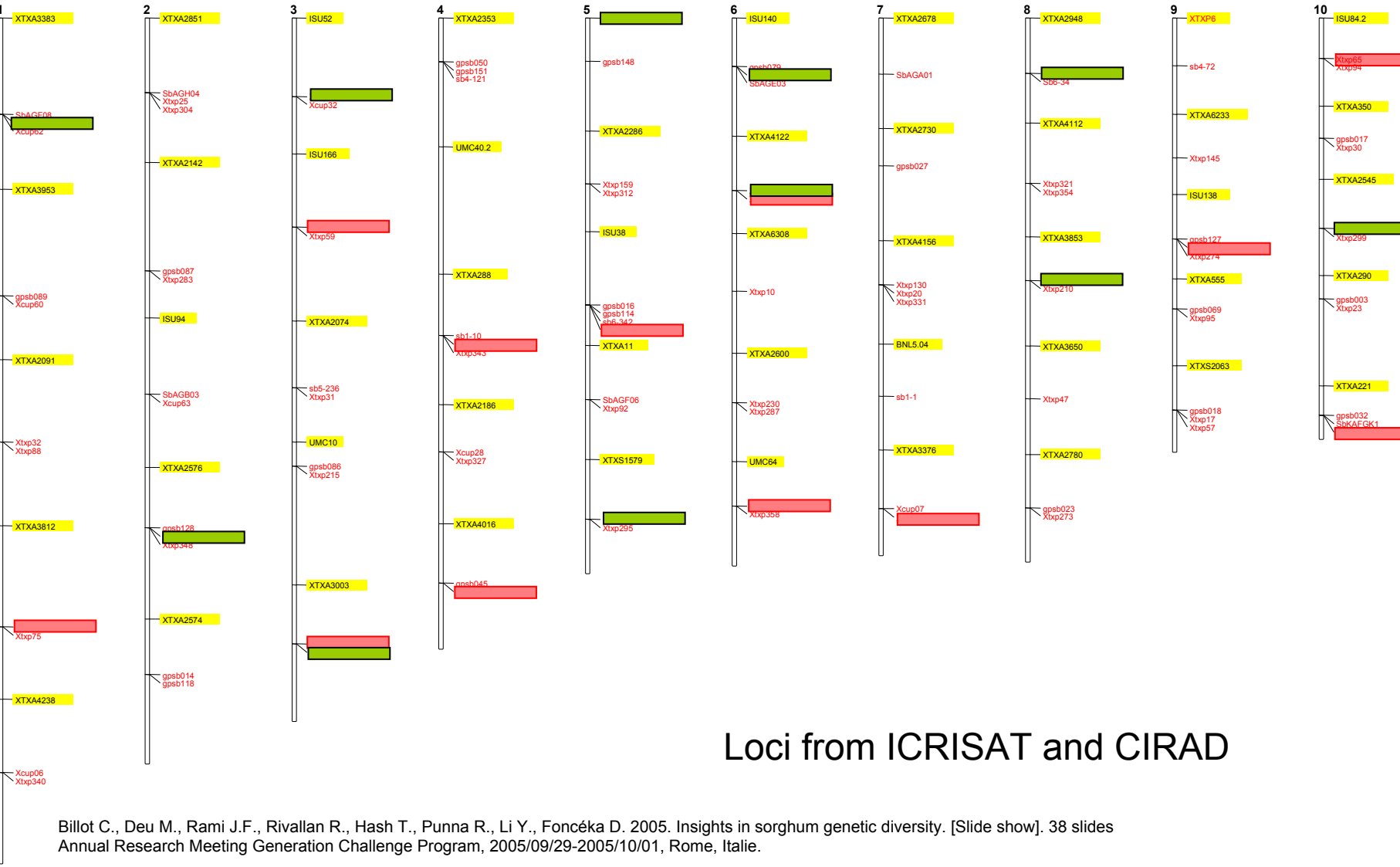
34 loci selected

CAAS (11)
CIRAD (11)
ICRISAT (12)

21 out of 49 uncovered bins
3 bins with 2 markers
1 chromosome with 1 marker only

Billot C., Deu M., Rami J.F., Rivallan R., Haeb T., Punna R., Li Y., Folkeéka D., 2005. Insights in sorghum genetic diversity. [Slide show]. 38 slides Annual Research Meeting Generation Challenge Program, 2005/09/29-2005/10/01, Rome, Italie.

20 loci analyzed



Loci from ICRISAT and CIRAD

Billot C., Deu M., Rami J.F., Rivallan R., Hash T., Punna R., Li Y., Foncéka D. 2005. Insights in sorghum genetic diversity. [Slide show]. 38 slides Annual Research Meeting Generation Challenge Program, 2005/09/29-2005/10/01, Rome, Italie.

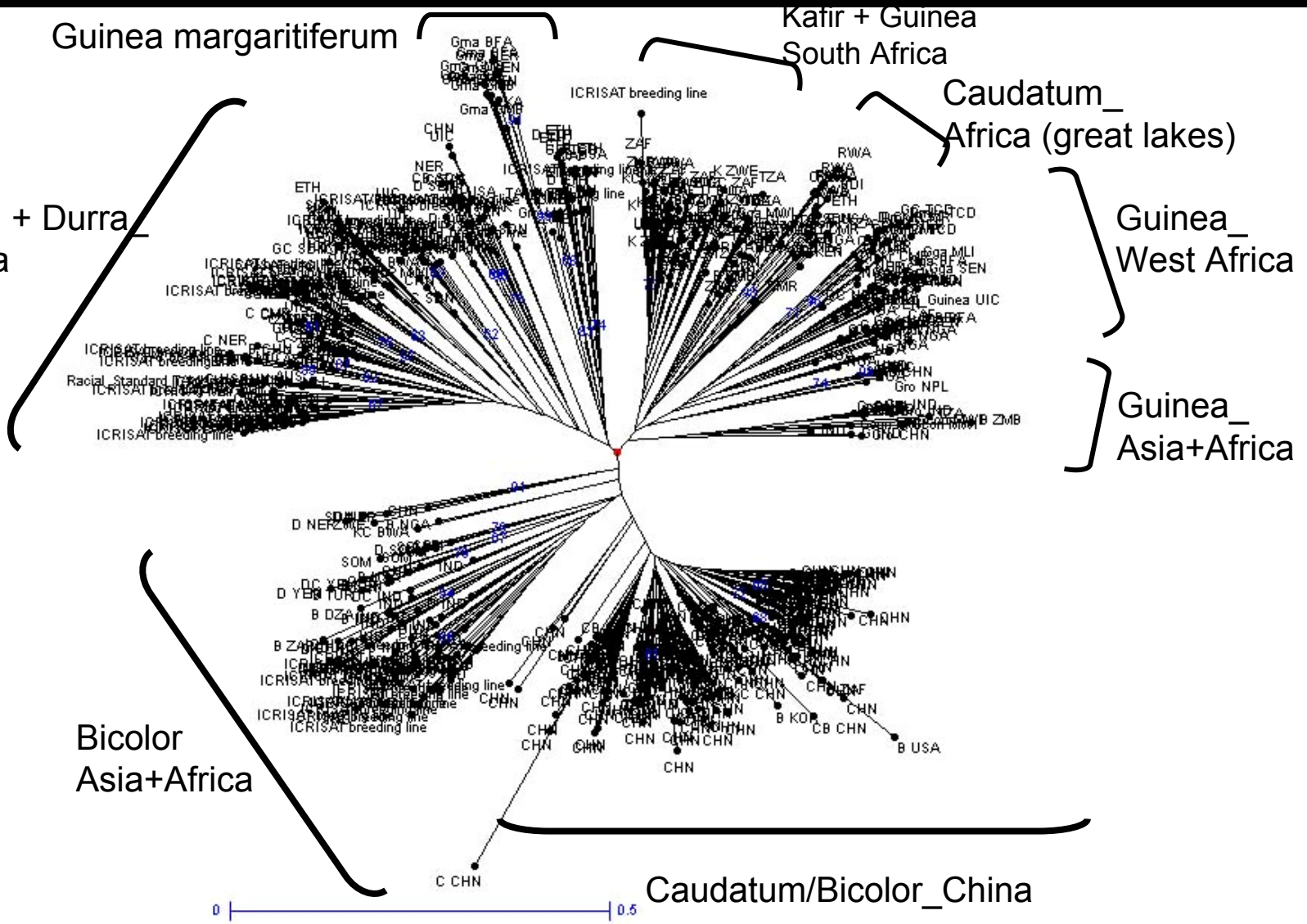
Diversity Analysis

- 661 accessions
- 20 loci
  - 8 Dinucleotides
  - 10 Trinucleotides
  - 2 Tetranucleotides
- 272 alleles
  - Mean # alleles / Locus = 13.6
  - between 4 and 30 alleles / locus
  - No correlation between type and Na
  - High number of rare alleles
    - 199 alleles below 0.05%
    - 132 alleles below 0.01%
- 651 genotypes
- LD same bin loci (Genetix)
  - Xcup61/Xcup11 = 0.052 ***
  - Xcup02/Xtxp339 = 0.032 ***

  Not higher than between other loci
- Simple matching dissimilarity index (Darwin)
  - NJ analysis (bootstrap, 1000 replications)
  - Factorial analysis

Billot C., Deu M., Rami J.F., Rivallan R., Hash T., Punna R., Li Y., Foncéka D. 2005. Insights in sorghum genetic diversity. [Slide show]. 38 slides Annual Research Meeting Generation Challenge Program, 2005/09/29-2005/10/01, Rome, Italie.

Neighbor Joining Analysis



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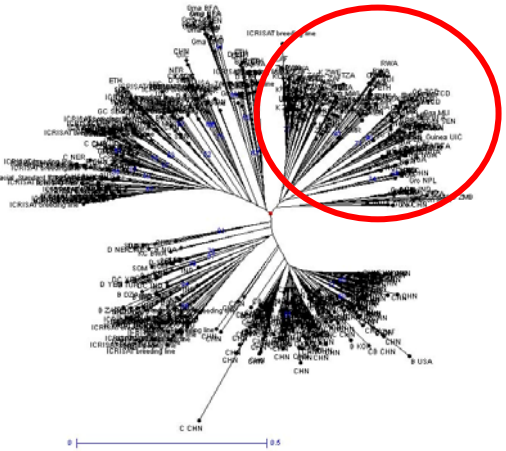
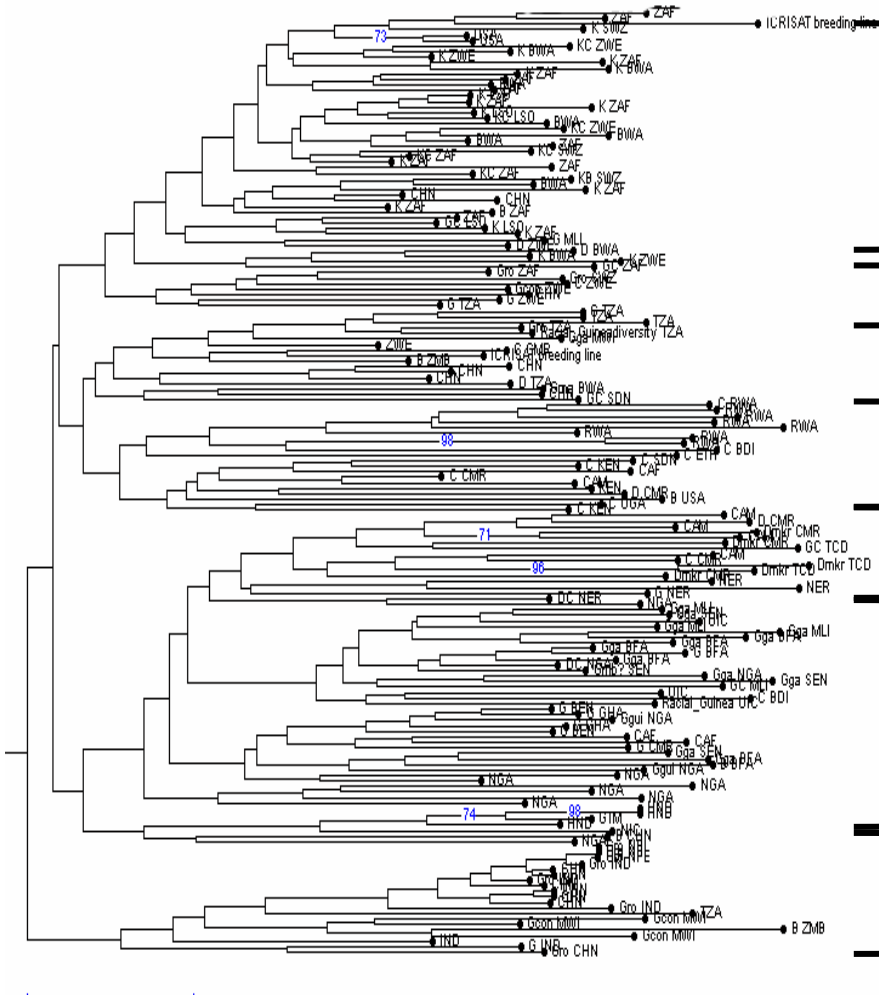
ophenetic r=0.9

Neighbor Joining Tree





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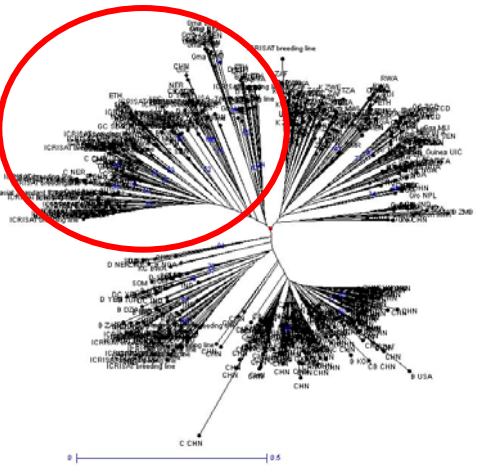
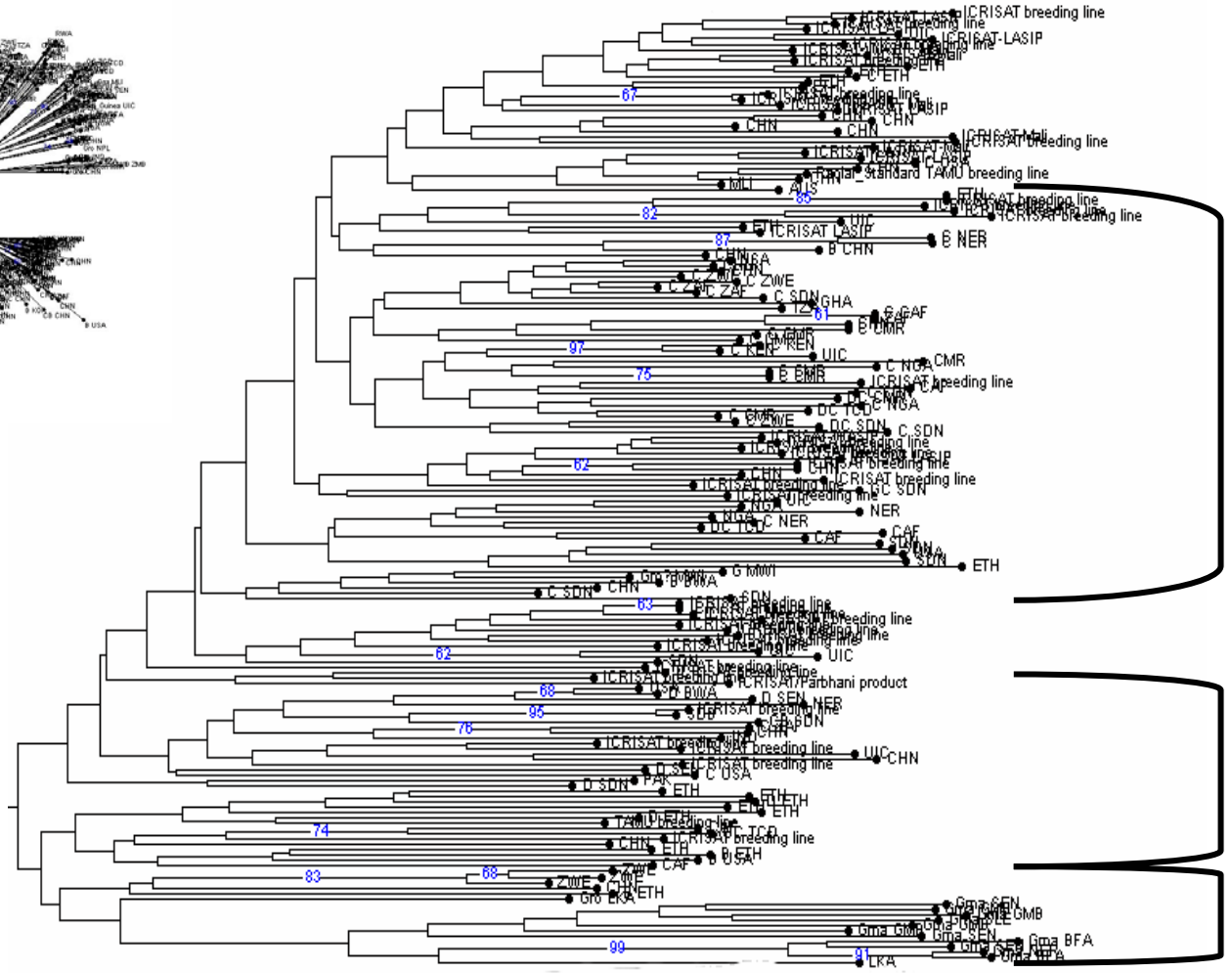
Neighbor Joining Tree





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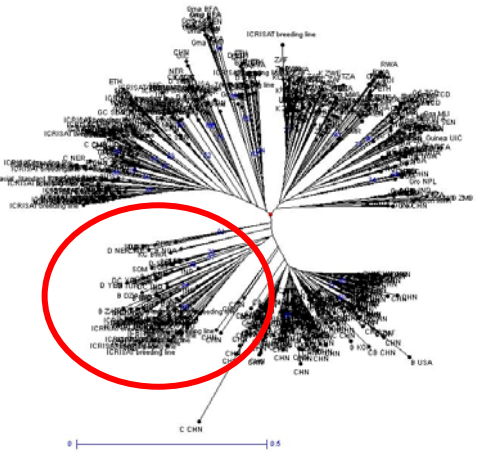

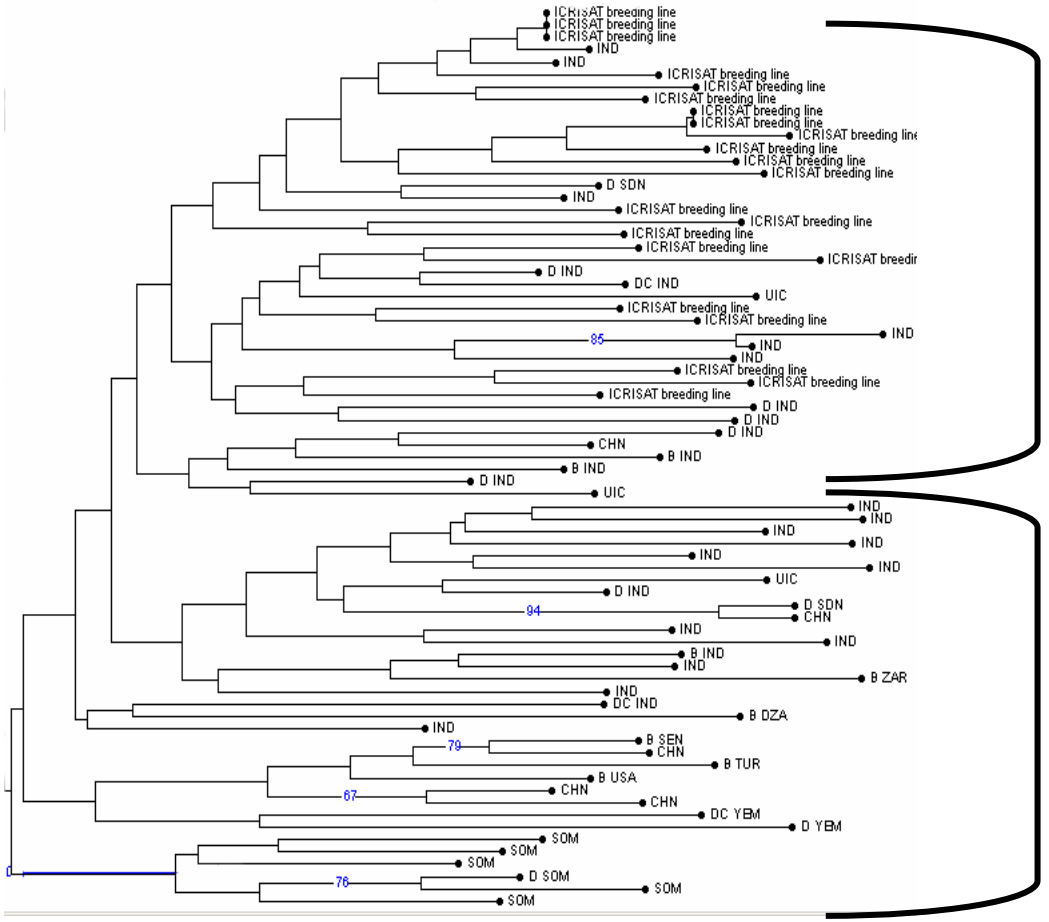

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Factorial analysis

- High weigth induced by high number of Chinese accessions
- Redundancy (breeding lines)

- New data set
  - 399 accessions x 20 loci

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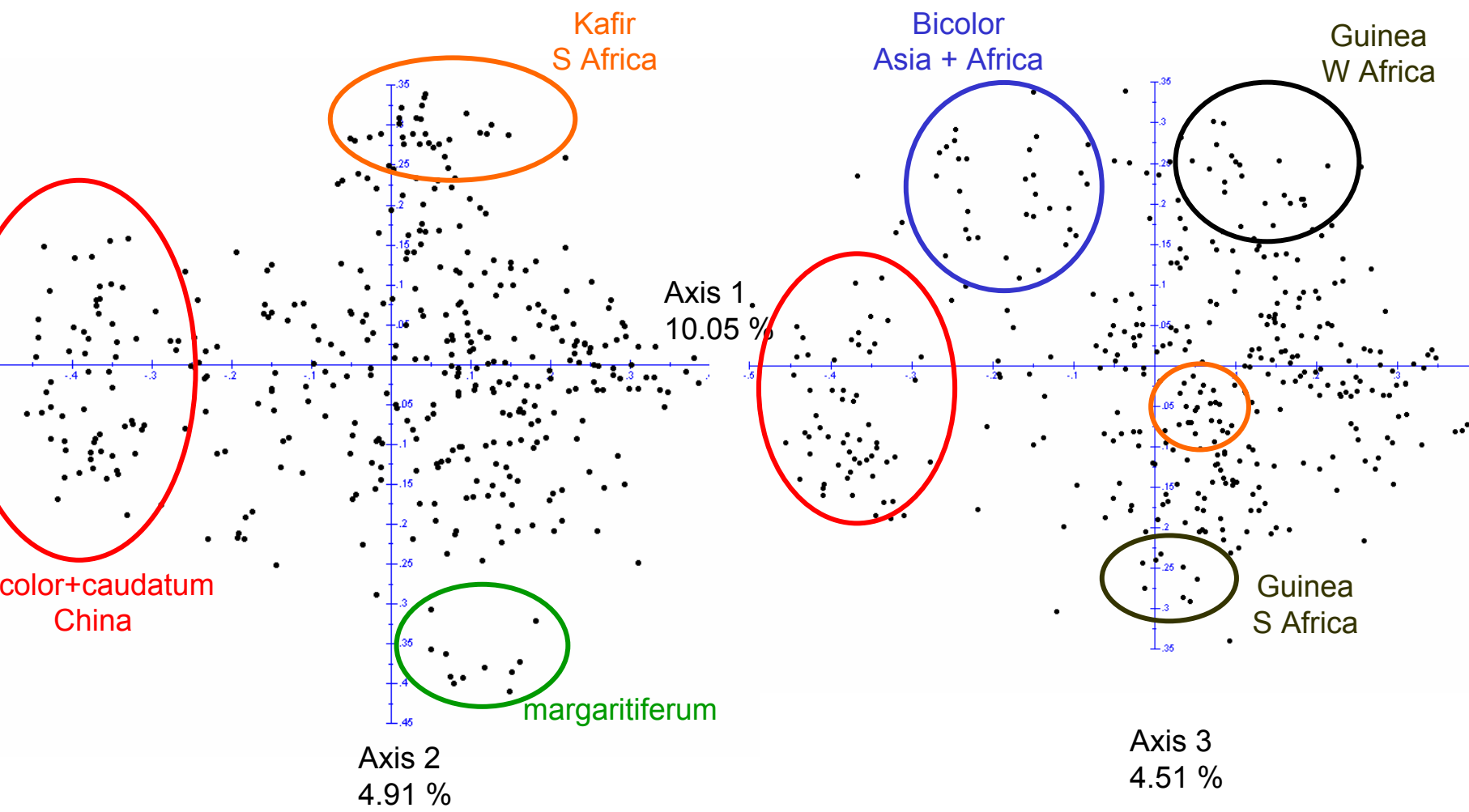
Factorial Analysis



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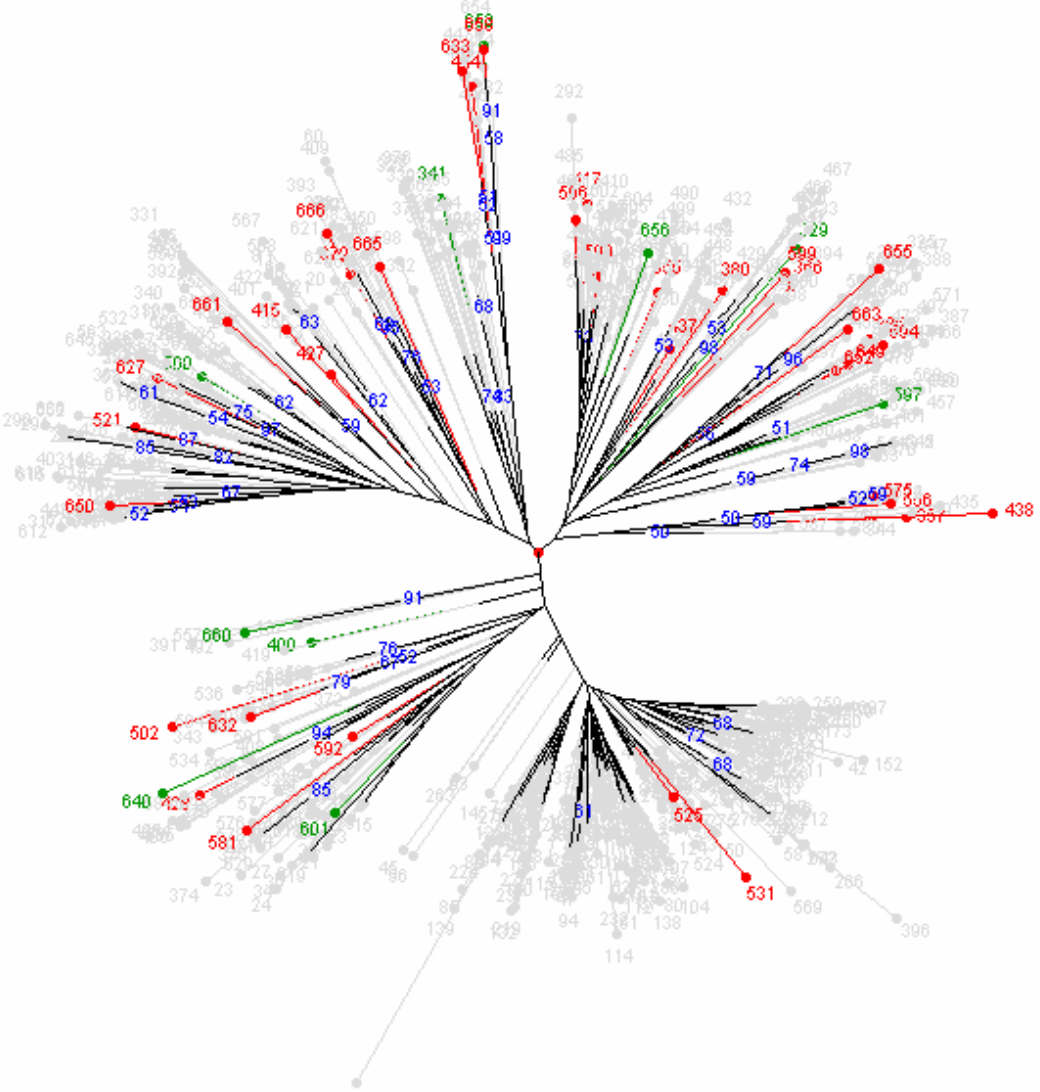
Validation of the choice of the 1st set

48 accessions
Controls



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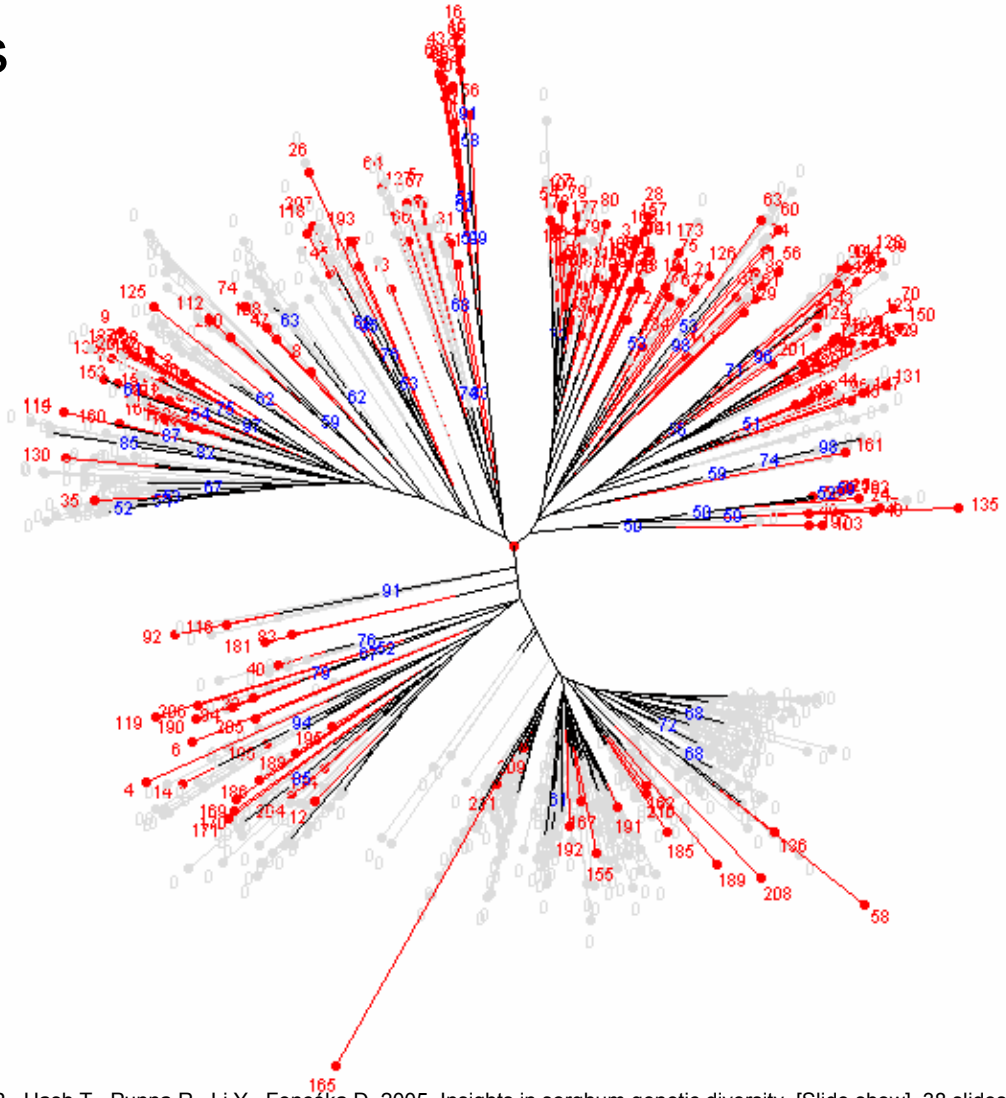
Comparison with previous data

205 accessions



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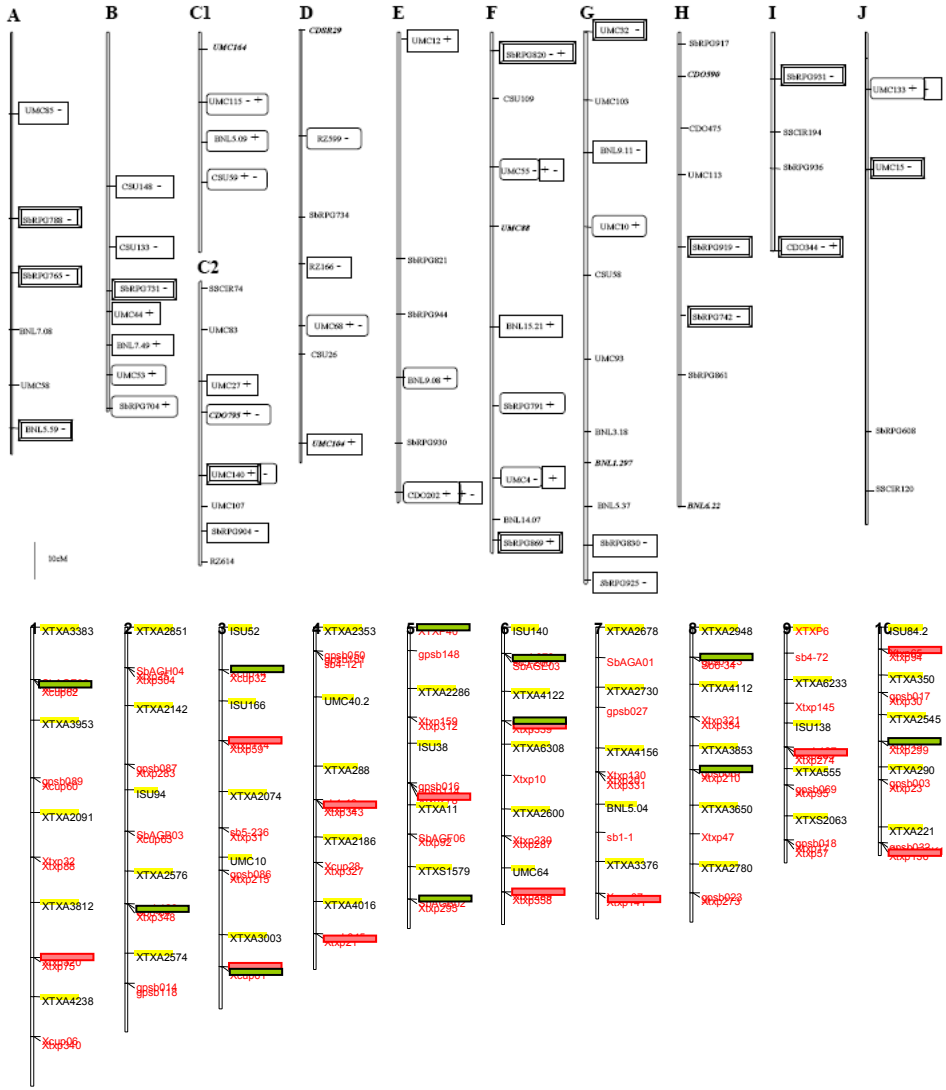
Comparison with previous data

- 74 RFLP probes
  - 219 polymorphic bands
  - 194 genotypes
- 20 SSR loci
  - 224 alleles
  - 203 genotypes



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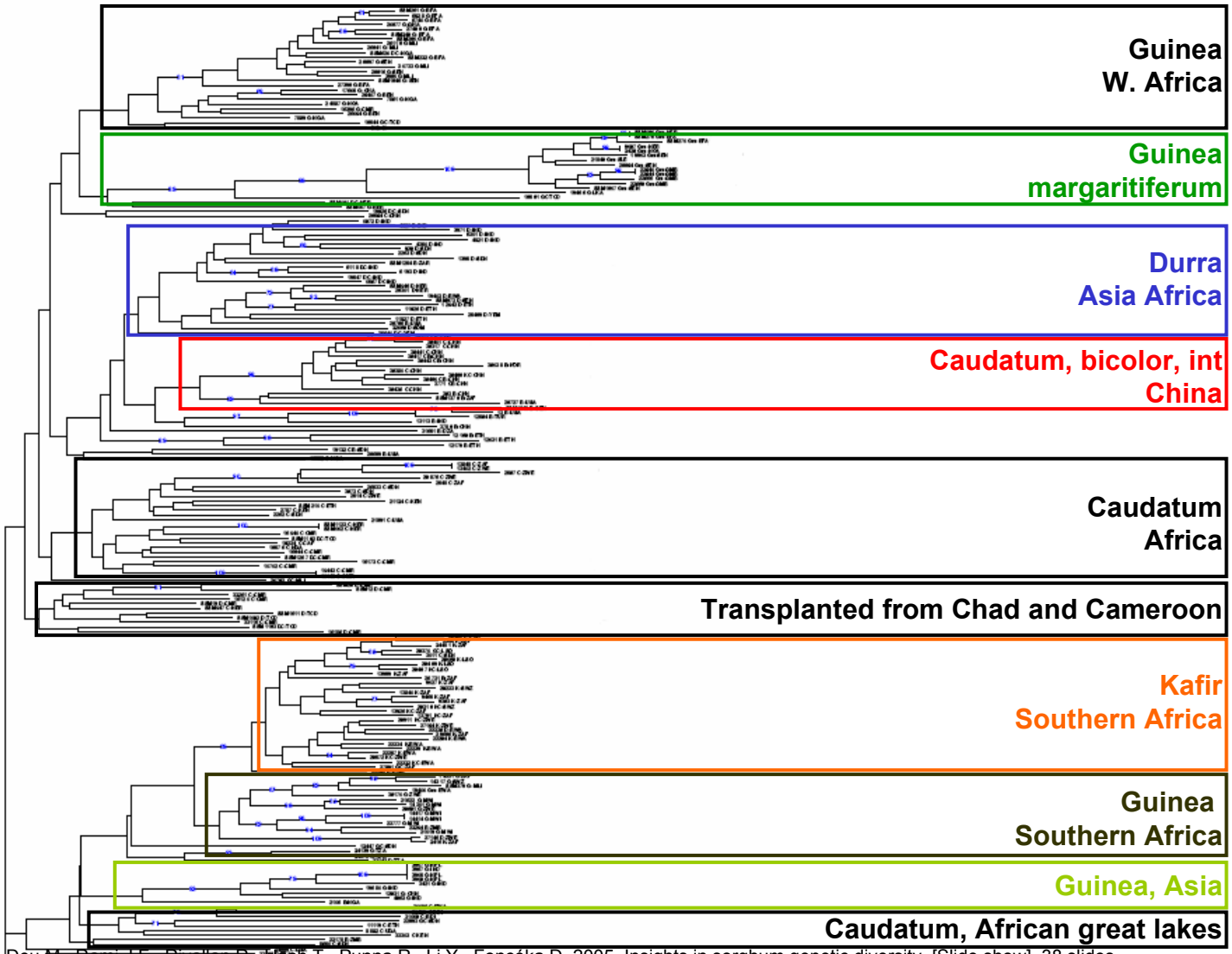
Neighbor Joining Tree - RFLP



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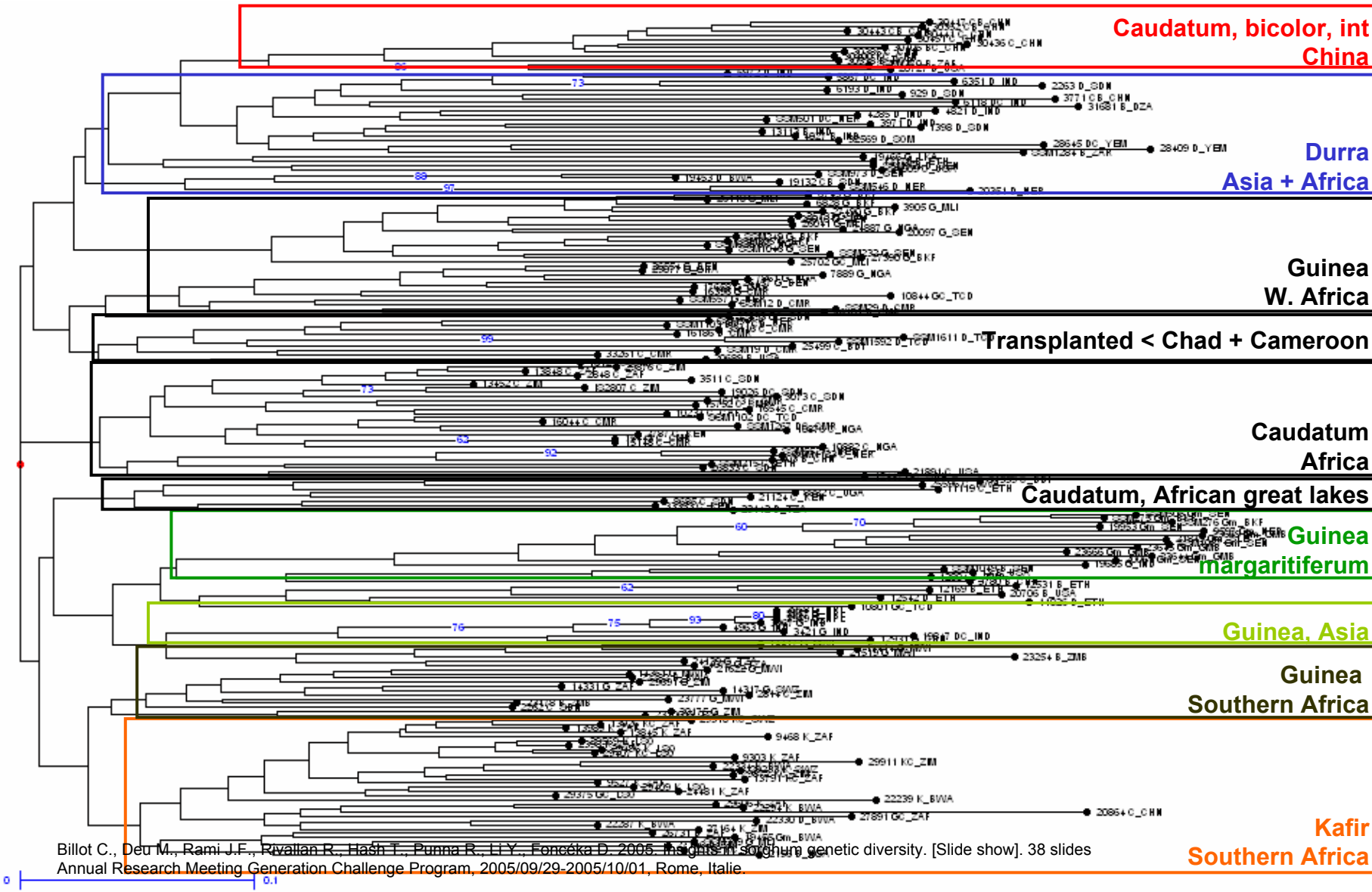
Neighbor Joining Tree - SSR



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Comparing the 2 trees

- Different topologies
  - Cophenetic correlation
    - SSR r=0.89
    - RFLP r=0.92
  - Comparison
    - Larger distance assessed by SSR (mutation pattern ?)
    - Distance between the 2 trees = 0.41
    - No good congruence between trees
    - Maximum agreement Sub-Tree: 51 accessions conserved only

- SSR NJ tree
  - Guinea margaritiferum
  - “Biphiletic” origin of guinea from southern and western Africa, not detected with RFLP

- More SSR markers to be run

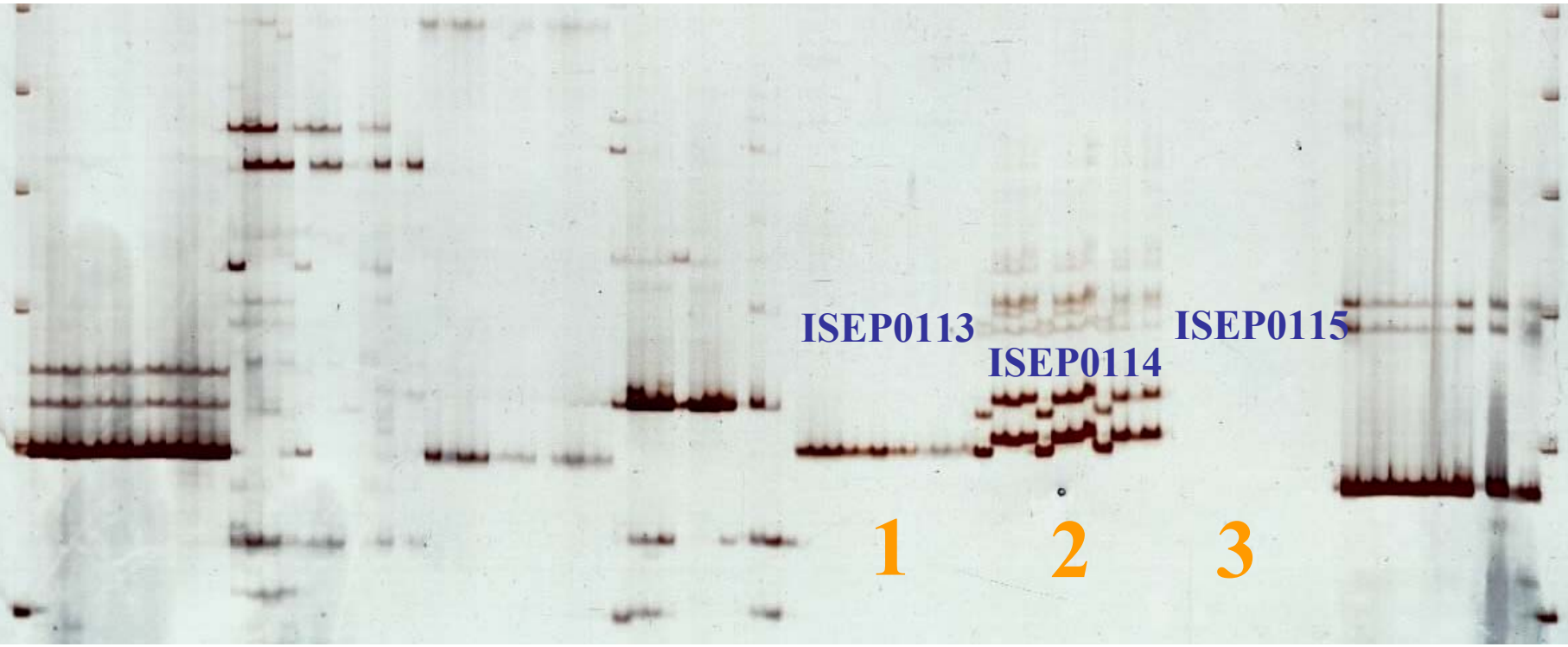
Billot C., Deu M., Rami J.F., Rivallan R., Hash T., Punna R., Li Y., Foncéka D. 2005. Insights in sorghum genetic diversity. [Slide show]. 38 slides Annual Research Meeting Generation Challenge Program, 2005/09/29-2005/10/01, Rome, Italie.

Development of new markers

- EST-derived SSR (ICRISAT)
  - From 90K sorghum EST sequences in GenBank identified circa 9K containing SSR repeats
  - BLAST against each rice chromosome
  - Selected 50 sorghum EST-SSR candidates distributed across each of 12 rice LGs
  - Design primer pairs, optimize PCR conditions, and check polymorphism
  - Start mapping ***Xisep*** series EST-SSR loci

Billot C., Deu M., Rami J.F., Rivallan R., Hash T., Punna R., Li Y., Foncéka D. 2005. Insights in sorghum genetic diversity. [Slide show]. 38 slides Annual Research Meeting Generation Challenge Program, 2005/09/29-2005/10/01, Rome, Italie.

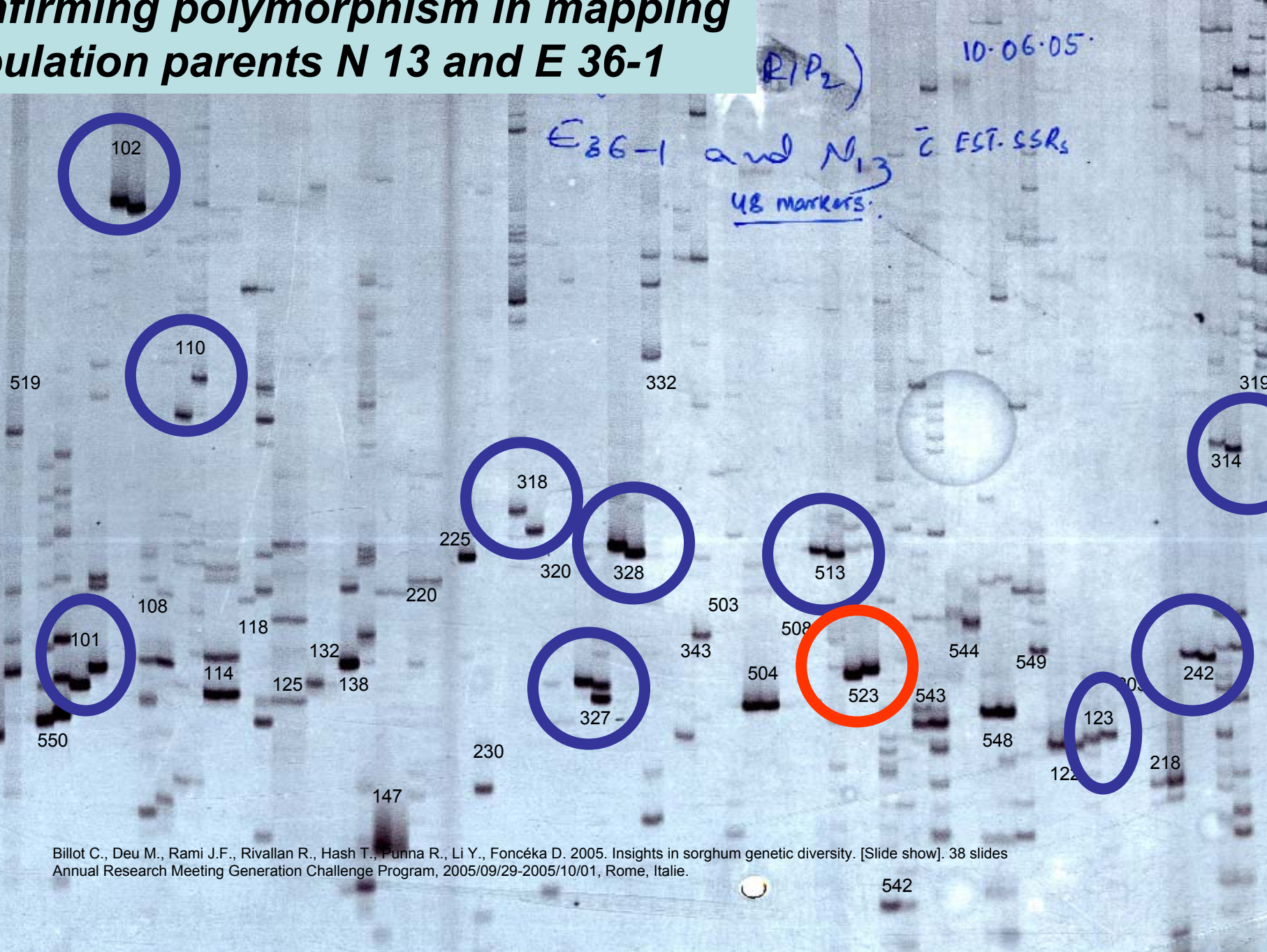
Optimization of EST-SSR markers in Sorghum - 1



1 = Monomorphic **2 = Polymorphic** **3 = No amplification**

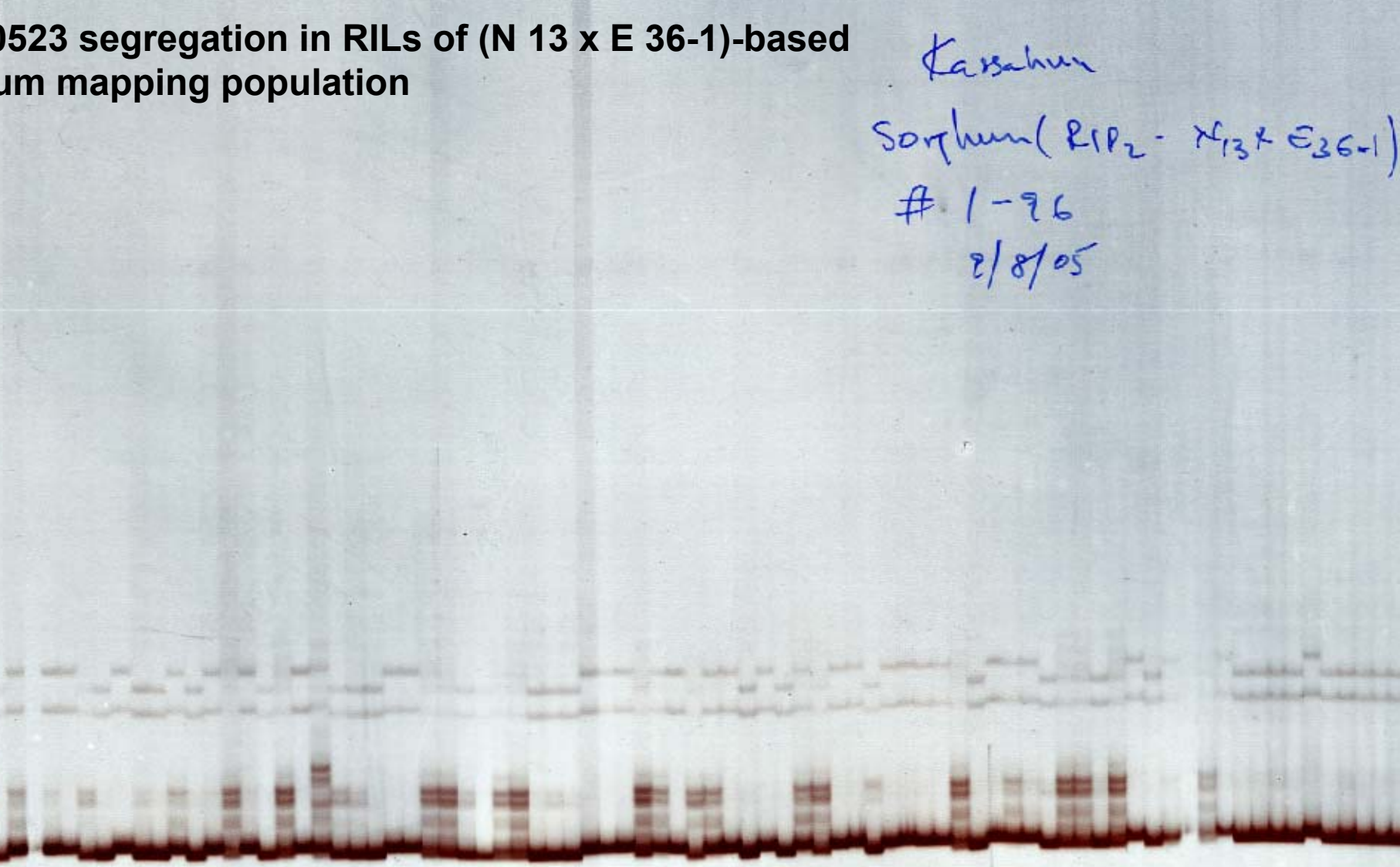
Billot C., Deu M., Rami J.F., Rivallan R., Hash T., Punna R., Li Y., Foncéka D. 2005. Insights in sorghum genetic diversity. [Slide show]. 38 slides Annual Research Meeting Generation Challenge Program, 2005/09/29-2005/10/01, Rome, Italie.

...firming polymorphism in mapping ...ulation parents N 13 and E 36-1



Billot C., Deu M., Rami J.F., Rivallan R., Hash T., Punna R., Li Y., Foncéka D. 2005. Insights in sorghum genetic diversity. [Slide show]. 38 slides Annual Research Meeting Generation Challenge Program, 2005/09/29-2005/10/01, Rome, Italie.

0523 segregation in RILs of (N 13 x E 36-1)-based um mapping population



Billot C., Deu M., Rami J.F., Rivallan R., Hash T., Punna R., Li Y., Foncéka D. 2005. Insights in sorghum genetic diversity. [Slide show]. 38 slides Annual Research Meeting Generation Challenge Program, 2005/09/29-2005/10/01, Rome, Italie.

Development of new markers

- neutral SSR (CIRAD)
  - Developed from enriched library (1042 nex sequences)
  - 600 new putative markers
  - 89 tested
  - 47 new markers, maped
  - Cover bins for which no marker were available

Billot C., Deu M., Rami J.F., Rivallan R., Hash T., Punna R., Li Y., Foncéka D. 2005. Insights in sorghum genetic diversity. [Slide show]. 38 slides Annual Research Meeting Generation Challenge Program, 2005/09/29-2005/10/01, Rome, Italie.

Further developments

- Still need to be done for the GCP-04
  - Check the data for the remaining 14 loci over the 661 accessions (mainly “CAAS” loci)
  - Complete data analysis
  - Choice of 20 markers from newly developed and “older” markers, no genotyping for year “2004”

- To be done for the GCP-05
  - Genotyping of the 2300 accessions
  - Data analysis of the complete dataset

Billot C., Deu M., Rami J.F., Rivallan R., Hash T., Punna R., Li Y., Foncéka D. 2005. Insights in sorghum genetic diversity. [Slide show]. 38 slides Annual Research Meeting Generation Challenge Program, 2005/09/29-2005/10/01, Rome, Italie.